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Distribution of Fungal Genera in Stockpiled Topsoil and Coal Mine Spoil Overburden

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Abundances, genera diversity, and similarity coefficients of soil fungi were determined for stockpiled topsoil and spoil overburden at a surface coal mine in New Mexico and were compared to an adjacent undisturbed reference soil. Although the diversity of fungal genera in stockpiled topsoil was somewhat less than in the undisturbed soil, the two were more similar in fungal genera diversity and in the types of fungi isolated than was the spoil overburden material.

Keywords: Mine spoil, reclamation, soil microorganisms, diversity

Introduction

Topsoil is the natural surface material that occurred on a site before surface mining began. During strip-mining, the topsoil, which normally includes the A (if present) and part or all of the B horizon is segregated, removed, and stockpiled. The overburden material (the material above the coal seam) is then removed by dragline, and is piled in a series of windrow banks. Later the overburden is leveled, and the stored topsoil is respread 20 to 30 cm deep over the overburden material to provide a planting medium.

Preliminary assessments have indicated that topsoil stored for years, and especially spoil overburden material, has little biological resemblance to the undisturbed surface soil (Fresquez and Lindemann 1982, Miller and Cameron 1976, Miller et al. 1979, Rives et al. 1980, USDA Forest Service 1979).

Fresquez and Lindemann (1982) reported fewer microorganisms and a reduced diversity of fungal genera in these soil and spoil materials compared to an undisturbed reference soil. However, only relative fungal genera distributions were presented. The relative diversity of fungal genera usually is based only on the presence or absence of a representative fungal colony. The objective of this study was to present a more complete autecological description of the abundance and diversity of fungal genera in stockpiled material and spoil overburden and relate these values to that of undisturbed soil.

Materials and Methods

The soil and spoil samples used in this study were from the San Juan coal strip-mine near Farmington, N. Mex. Soils vegetation, and environmental parameters of this area have been described previously (Fresquez and Lindemann 1983, Fresquez et al. 1983).

Five composite soil samples were collected from a spoil overburden pile (approximately 2 to 3 years old), a topsoil stockpile (approximately 3 to 4 years old), and an adjacent undisturbed site. For each composite sample, 10 subsamples were collected randomly to a depth of 13 cm using a hand probe. The 10 subsamples were mixed thoroughly in a clean plastic bag, were placed in a sterile bag, and immediately were cooled in an ice chest for transport to the laboratory. At the laboratory the composite samples were passed through a 2-mm sieve, and were stored at 4°C before microbial enumeration. A subsample from each composite was taken for laboratory analysis at the New Mexico State University Soil and Water Testing Laboratory. All methods of

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nalysis have been described previously (Fresquez and Lindemann 1983). The chemical and physical properties of the materials are given in table 1.

Numbers of fungi were estimated by the dilution and plating technique (Clark 1965), except that a 0.85% saline solution was used as a diluent. Dilutions were plated in triplicate on rose bengal-streptomycin agar (Martin 1950) and were incubated at 30°C for 4 days. The number of fungi were reported on a per gram of oven-dry soil basis. Fungi were isolated by evenly distributing 1 ml of a 10^{-3} dilution (from a 10-g oven-dry weight equivalent soil sample) over the surface of solidified rose bengal-streptomycin agar. Six plates were inoculated for each composited site sample and were incubated at 30°C for 7 days. Fungi were classified by genus using the taxonomic guides of Barnett and Hunter (1972), Barron (1968), and Gilman (1968).

Variations in the number of fungal propagules between the undisturbed soil, the spoil overburden material, and the stockpiled topsoil material were analyzed by Dunnett's Pairwise Multiple Comparison Test (Dunnett 1980) at the 5% probability level.

The following four indexes of diversity were employed to compare the distribution patterns for each of the genera groups isolated. First was Shannon's index of species diversity (Zar 1974), which estimates community richness as:

$$H = -\sum_{i=1}^{k} P_i \log P_i \qquad (1)$$

where P_i = the proportion of genera i in the sample, and k = the number of genera; the corresponding test for evenness is:

$$J = H/H_{max} \qquad (2)$$

where H_{max} = maximum possible diversity.

An estimate of the similarity in the general composition among the sample populations was calculated with Sorensen's presence community coefficient (SPCC), which was described by Mueller-Dombois and Ellenberg (1974) as:

$$SPCC = 200C/A + B \qquad (3)$$

where C is the total number of genera common to two samples, A is the total number of genera in sample A, and B is the total number of genera in sample B. If the same genera were found in both samples, then the community coefficient would be 100, whereas if they had no genera in common, the coefficient would be 0.

Sorensen's quantitative similarity coefficient (SQSC), which estimates the similarity in the relative abundance of genera between samples, also was calculated. The SQSC is described by Mueller-Dombois and Ellenberg (1974) as:

$$SQSC = 200Mw/M_A + M_B \qquad (4)$$

where Mw refers to the total of the smaller quantitative values of the genera common to two samples, M_A is the total number of individual isolates in sample A, and M_B is the total number of individual isolates in sample B.

Results and Discussion

Numbers of fungal propagules were not significantly different among the undisturbed soil, the stockpiled topsoil material, or the overburden material. The overburden material, however, contained the lowest numbers of fungal propagules.

Table 1.—Characteristics of an undisturbed soil and disturbed soil and spoil materials from the San Juan coal strip-mine.

Soil parameter[1]	Undisturbed soil	Stockpiled topsoil	Overburden spoil
EC[2]	0.56	2.64	9.72
SAR	1.12	6.75	27.58
pH	7.83	7.72	7.13
Ca	4.37	8.95	16.95
Mg	0.95	2.85	3.76
Na	1.83	16.39	88.75
K	0.60	0.82	1.33
P	4.44	4.95	6.00
SO_4	0.73	34.35	100.77
NH_4	3.40	2.40	4.40
NO_3	0.10	0.10	10.10
Total N	247	305	1120
Organic matter	0.54	0.64	4.35
CEC	3.14	4.06	15.10
Texture	Loamy sand	Sandy loam	Clay

[1] *Water-soluble cations, pH, EC and SO_4 determined from saturated paste extract. Soil P, NH_4^+– N, and NO_3^-– N determined from 1:5 water extract. Organic matter determined by the Walkley-Black method, and total N determined by the macro-Kjeldahl method.*

[2] *EC is expressed as mmhos/cm; Ca, Mg, Na, K, and SO_4 are expressed as meq/L; P, NH_4, NO_3, and total N are expressed as ppm; organic matter is expressed as percent; and CEC is expressed as meq/100g.*

Diversity indexes can be used to express the degree of soil physiochemical stress placed on the soil biological community. The undisturbed soil was characterized by a higher fungal genera diversity (H) and evenness (J) index, than either the stockpiled topsoil or overburden materials (table 2). Generally, a high diversity of species or genera is indicative of a stable and well-balanced soil ecosystem, while unstable or stressed soil ecosystems are dominated by only a few species or genera (Atlas and Bartha 1981, Jernelov and Rosenberg 1976). The stockpiled topsoil material, which was represented by 11 fungal genera, had a higher diversity index than the spoil material. Only four fungal genera were representative of the spoil material, with *Penicillium* and *Chrysosporium* comprising 88% of the total colonies isolated; these probably were the genera most responsible for most of the enumerated propagules.

Sorensen's community (SPCC) and similarity (SQSC) coefficients indicated that the stockpiled topsoil material was microbially more similar to the undisturbed reference soil than was the overburden spoil material:

Site	SPCC	SQCC
Stockpiled topsoil	47	38
Overburden spoil	26	28

Conclusions

The similarity in the number of fungal propagules between the stockpiled topsoil material and the undisturbed soil, and the higher fungal genera diversity and similarity coefficients in both of these materials, when compared to the spoil material, suggest that the topsoil material was a biologically more active medium than was the spoil overburden material. The low fungal genera diversity, the dominance of only a few microfungal genera, and the very low similarity in the types of fungal genera isolated in the spoil overburden material compared to the undisturbed reference soil may indicate extreme and adverse soil chemical and physical properties of the overburden material.

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Table 2.—Distribution of fungal genera in an undisturbed soil, and disturbed soil and spoil materials from the San Juan coal strip-mine.[1]

Fungal groups	Undisturbed soil	Stockpiled topsoil	Overburden spoil
Acremonium	1	0	0
Alternaria	1	0	2
Annellophorella	1	0	0
Aspergillus	5	13	1
Chaetomium	2	10	0
Chrysosporium	0	0	9
Cladosporium	0	1	0
Curvularia	15	3	0
Dreshlera	1	1	0
Fusarium	7	10	0
Humicola	5	0	0
Microascus	0	2	0
Myrothecium	1	0	0
Mortiorella	1	0	0
Mycelia sterilia	0	1	0
Penicillium	15	44	14
Phoma	5	0	0
Rhizopus	5	0	0
Sepedomium	0	1	0
Stachybotrys	3	1	0
Thielavia	1	0	0
Trichoderma	3	0	0
Unidentified			
Isolate #1	15	0	0
isolate #2	3	0	0
Total no. of isolates	90	87	26
Total no. of genera	19	11	4
Fungal genera diversity	1.091	0.689	0.444
Evenness	0.853	0.662	0.738

[1] *Relative density of isolates from six 1:1000 soil dilution plates.*

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